Impact of Small-Quantity Lipid-Based Nutrient Supplement on Hemoglobin, Iron Status and Biomarkers of Inflammation in Pregnant Ghanaian Women[1-7]

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[5]Abbreviations: AGP, Alpha-1 glycoprotein; CRP, C-reactive protein; EFA, Essential Fatty Acid; GA, Gestational age; GW, Gestational weeks; IDA, Iron Deficiency Anemia; IFA, Iron and folic acid; ; LNS, Lipid-based Nutrient Supplement; MMN, Multiple Micronutrients; SQ-LNS, Small-quantity lipid-based nutrient supplement; TfR, Transferrin receptor; ZPP, Zinc Protoporphyrin.

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Contributor statement

SA-A, AL, PA, MZ, SV, and KGD designed the research; MZ was responsible for the development and production of the LNS used in the study based on the specifications agreed

upon by the iLiNS Project; SA-A, AL, and HO conducted the research; LMB and BO performed laboratory analysis; SA-A performed the statistical analysis; AL, PA, and KGD advised on the analysis; SA-A and KGD wrote the manuscript; and AL, HO, PA, MZ, LMB, BO, and SV reviewed the draft manuscript. All authors read and approved the final manuscript.

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Abstract

We examined hemoglobin (Hb, g/L), iron status (zinc protoporphyrin, ZPP, μmol/mol heme, and transferrin receptor, TfR, mg/L), and inflammation (C-reactive protein, CRP and alpha-1 glycoprotein, AGP) in pregnant Ghanaian women who participated in a randomized controlled trial. Women (n=1320) received either 60 mg Fe + 400 μg folic acid (IFA); 18 micronutrients including 20 mg Fe (MMN); or small-quantity lipid-based nutrient supplements (SQ-LNS, 118 kcal/d) with the same micronutrient levels as in MMN, plus 4 additional minerals (LNS) daily during pregnancy. Intention-to-treat analysis included 349, 354, and 354 women in the IFA, MMN and LNS groups, respectively, with overall baseline mean Hb and anemia (Hb <100) prevalence of 112 and 13.3%, respectively. At 36 gestational weeks, overall Hb was 117 and anemia prevalence was 5.3%. Compared with the IFA group, the LNS and MMN groups had lower mean Hb (120±11 vs 115±12 and 117±12, respectively; p<0.001), higher mean ZPP (42±30 vs 50±29 and 49 ±30; p=0.010) and TfR (4.0±1.3 vs 4.9±1.8 and 4.6±1.7; p<0.001), and greater prevalence of anemia (2.2% vs 7.9% and 5.8%; p=0.019), elevated ZPP (>60) [9.4% vs 18.6% and 19.2%; p=0.003] and elevated TfR (>6.0) [9.0% vs 19.2% and 15.1%; p=0.004]. CRP and AGP concentrations did not differ among groups. We conclude that among pregnant women in a semi-urban setting in Ghana, supplementation with SQ-LNS or MMN containing 20 mg iron resulted in lower Hb and iron status but had no impact on inflammation, when compared with iron (60 mg) plus folic acid (400 μg). The amount of iron in such supplements that is most effective for improving both maternal Hb/iron status and birth outcomes requires further evaluation. This trial was registered at ClinicalTrials.gov as: NCT00970866.

Keywords lipid-based nutrient supplements, LNS, prenatal supplementation, multiple micronutrients, hemoglobin, iron status, inflammation

Introduction

Poor nutrient intake during pregnancy has been associated with several adverse consequences. It is estimated that up to 50% of the anemia among pregnant women in many developing country settings is due to iron deficiency (van den Broek et al., 1998) usually as a result of low dietary iron intake (World Health Organization, 1992) and poor iron bioavailability due to over-reliance on plant-based diets high in inhibitors of iron absorption such as phytate (Tatala et al., 1998). Consequences of anemia include reduced work capacity and increased risk of mortality for the mother, and premature delivery, low birth weight and poor mental development for the infant (Ren et al., 2007, International Anemia Consultative Group (INACG), 2002). While normal pregnancy is found to be associated with an increased inflammatory response (Picklesimer et al., 2008, Sacks et al., 1998), this response may be modified by macro- or micro-nutrients (Roberts et al., 2003). Higher intakes of folic acid (Bertran et al., 2005) and vitamin B_6 (Friso et al., 2001) have been associated with lower concentration of C-reactive protein (CRP), a common biomarker of inflammation. Several other dietary factors including essential fatty acids, EFAs (Rallidis et al., 2003) and antioxidants (Brighenti et al., 2005, Devaraj and Jialal, 2000) are also associated with the reduction of CRP concentration. Elevated CRP concentration in pregnancy is related to the development of pre-eclampsia and preterm delivery (Elovitz, 2006).

A major recommendation for increasing nutrient intake among pregnant women in developing countries is the one developed by WHO (WHO), which is the consumption of iron/folic acid (IFA) supplements containing 30-60 mg iron and 400 µg folic acid. In meta-analyses, this strategy, compared with no iron or placebo, reduced the risk of maternal anemia by 69-70 % (Imdad and Bhutta, 2012, Pena-Rosas et al., 2012) and iron deficiency by 57% (Pena-

Rosas et al., 2012), but appeared to increase the risk of reported side effects (relative risk (RR) = 2.36; 95% CI: 0.96 -5.82) particularly at iron doses of 60 mg or higher (Pena-Rosas et al., 2012). The WHO/UNICEF/UNU UNIMMAP (United Nations International Multiple Micronutrient Preparation) formulation containing 15 vitamins and minerals was more recently developed (UNICEF/WHO/UNU) to combat other possible deficiencies, e.g. for vitamins A, C and B_{12}, which may also contribute to anemia (World Health Organization, 1992). The dose of iron in the UNIMMAP was set at 30 mg (below 60 mg) for the following reasons: (a) the presence of vitamins A, B_2 and C in the UNIMMAP would enhance the absorption and utilization of iron, and therefore the lower amount of iron should be sufficient, (b) a lower iron dose would be associated with less negative side effects and therefore better adherence, (c) including 60 mg of iron would mean including at least 30 mg of zinc (to avoid possible negative influence of iron on zinc absorption), bringing the total amount of metals to 90 mg, which is likely to increase negative side effects, and (d) UNIMMAP may be used in conjunction with additional iron /folic acid tablets in individual cases of more severe anemia (assuming it is caused by iron deficiency). Meta-analyses suggested that supplementation with UNIMMAP and similar products (mostly containing 11 or more micronutrients including 30 mg iron) had the same effect on maternal hemoglobin and iron status as iron (usually at 60 mg dose) with or without folic acid (Allen and Peerson, 2009, Haider et al., 2011), while also reducing the risk of low birth weight (Fall et al., 2009, Haider and Bhutta, 2012, Ramakrishnan et al., 2012).

Our group developed small quantity (20 g/d) lipid-based nutrient supplements (SQ-LNS) for pregnant and lactating women (Arimond et al., 2013) to provide micronutrients together with EFAs, using a minimum food base that supplies a small amount of energy (118 kcal/d) and high quality protein (2.6 g/d). In many populations, total energy intake among pregnant and lactating

women may be adequate, but the EFA content of the usual diet may be low (Michaelsen et al., 2011). The micronutrient composition of the SQ-LNS was generally based on the UNIMMAP formulation and a similar product used in Guinea Bissau (Kaestel et al., 2005), but we further reduced the daily iron dose to 20 mg, based on evidence that 20 mg day^{-1} may be an adequate dose to prevent iron deficiency anemia during pregnancy (even for women who are iron deficient at entry to prenatal care) and causes fewer gastrointestinal side effects, compared to higher doses of iron (Zhou et al., 2009). We estimated (Arimond et al., 2013) that in addition to iron coming from the usual diet, the 20 mg of iron from a daily supplement would meet the recommended dietary allowance (RDA) of 27 mg iron during pregnancy (and be close to the 30 mg/d dose in the UNIMMAP formulation) while not greatly exceeding the RDA (9 mg/d) for iron during lactation (IOM, 2001, Arimond et al., 2013).

Currently, there is a growing interest in the potential use of Small-Quantity Lipid-based Nutrient Supplements (SQ-LNS) among pregnant women in developing-country settings (Hambidge et al., 2014, Research Engagement on Food Innovation for Nutritional Effectiveness (REFINE), 2013), because of evidence suggesting a positive impact of the product on certain pregnancy outcomes (Adu-Afarwuah et al., 2015). However, little is known about the impact of SQ-LNS on maternal outcomes such as anemia, iron status and inflammation. We previously reported (Adu-Afarwuah et al., 2015), that compared to IFA and a multiple micronutrient (MMN) capsule with most of the same micronutrients as the SQ-LNS, the SQ-LNS promoted fetal growth in vulnerable women, particularly primiparas, whilst the occurrence of serious adverse events did not differ between the 3 groups. In the current analysis, we compare the effect of the 3 supplementation regimens (IFA, MMN and SQ-LNS), on maternal hemoglobin (Hb),

93 iron status, and two biomarkers of inflammation (CRP and alpha-1 glycoprotein, AGP) during

94 pregnancy.

Methods

Study setting, design, participants and blinding

The iLiNS DYAD study in Ghana was conducted in several adjoining semi-urban communities in the Yilo Krobo and the Lower Manya Krobo Districts about 70 km north of Accra, Ghana. Details of the study setting, participants, design, randomization and masking schemes, and other key procedures have been reported elsewhere (Adu-Afarwuah et al., 2015). In brief, the study was designed as a partially double-blind, parallel, individually randomized, controlled trial with three equal-size groups. Pregnant women attending usual ante-natal clinics in four main health facilities in the area between December 2009 and December 2011 completed a screening questionnaire if they were ≥18 years old, ≤20 weeks gestation (as determined by the antenatal clinics mostly by fundal height), and had an antenatal card complete with history and examination. Informed consent for the screening was obtained by trained study workers at the antenatal clinics. Following screening, women were excluded if the antenatal card indicated HIV infection, asthma, epilepsy, tuberculosis or any malignancy. Additional exclusion criteria were known milk or peanut allergy, not residing in the area, intention to move within the next two years, unwillingness to receive field workers or take study supplement, participation in another trial, or gestational age (GA) >20 weeks before completion of the enrolment process.

Women who passed the screening were visited in their homes, where details of the study were provided, and those willing to participate were recruited, after signing or thumb-printing informed consent. Recruited women remaining eligible underwent a baseline laboratory assessment after consent, and were immediately randomized to receive one of three treatments daily: (a) 60 mg iron plus 400 µg folic acid (hereafter, IFA supplement or group); (b) multiple micronutrient capsule containing 18 vitamins and minerals (including 20 mg iron) (hereafter,

MMN supplement or group); and (c) SQ-LNS with similar micronutrients as the MMN supplement, plus other minerals and macronutrients (hereafter, LNS supplement or group). Group allocations were developed by the Study Statistician at UC Davis using a computer-generated (SAS version 9.3) randomization scheme (in blocks of nine), and were placed in sealed, opaque envelopes. At each enrolment, a Study Nurse offered nine envelopes at a time, and the woman picked one to reveal the allocation. Allocation information was kept securely by the Field Supervisor and the Study Statistician only.

The compositions of the 3 supplements were reported previously (Adu-Afarwuah et al., 2015), as well as the considerations underlying the concentrations of the nutrients in the MMN and SQ-LNS (Arimond et al., 2013). Apart from iron which was kept at 20 mg/day in the MMN and SQ-LNS, the vitamin and mineral contents were either 1x or 2x the RDA for pregnancy, or in a few cases, the maximum amount that could be included in the supplement given technical and organoleptic constraints. The IFA and MMN supplements were provided as capsules in blister packs, and were intended to be consumed with water after a meal, one capsule per day throughout pregnancy. The LNS supplement was in 20-g sachets, and was intended to be mixed with any prepared food, one sachet per day throughout pregnancy. To maintain blinding, two individuals independent of the study placed color-coded stickers behind the blister packs (three different colors for IFA and three for MMN supplements) so that the capsules were known to the study team and participants only by the colors of the stickers. Laboratory staff and data analysts had no knowledge of group assignment until all preliminary analyses had been completed and the allocation codes were broken. The study was registered on ClinicalTrials.gov (Identifier: NCT00970866) and was approved by ethics committees of the University of California, Davis,

the Ghana Health Service, and the University of Ghana Noguchi Memorial Institute for Medical Research.

Procedures

We collected socio-demographic information at baseline, and determined GA mostly by ultrasound biometry (Aloka SSD 500, Tokyo, Japan). During follow-up, field workers visited women in their homes every two weeks, whereupon they delivered a fresh supply of supplement and monitored supplement intakes. At each of laboratory assessments at baseline and at 36 GW, women's weight (Seca 874) and height (Seca 217) were measured, and peripheral malaria parasitemia (Clearview Malarial Combo, Vision Biotech, South Africa), hemoglobin, Hb (HemoCue AG, Wetzikon, Switzerland) and zinc protoporphyrin, ZPP (hematofluorometer, Aviv Biomedical Co. NJ, USA), were determined using venous blood (Adu-Afarwuah et al., 2015). We used the original Aviv cover-slides and 3-level control material for the ZPP measurements, after red blood cells were washed three times with normal saline. Plasma samples obtained after blood was centrifuged at 1,252 x *g* for 15 min were stored in Ghana at -20 ºC, before being air-freighted on dry ice to UC Davis, where soluble transferrin receptor (TfR, mg/L), C-reactive protein (CRP, mg/L), and alpha-1 glycoprotein (AGP, g/L) concentrations were determined using a Cobas Integra 400 plus Automatic Analyzer (Roche Diagnostic Corp., Indianapolis, IN).

At 36 GW, the continuous outcomes measures were Hb (g/L), ZPP (µmol/mol heme), and plasma TfR (mg/L), CRP (mg/L), and AGP (g/L) concentrations, whilst the binary outcome measures were the percentages of women with low Hb, high Hb, and elevated ZPP, TfR, CRP and AGP.

Sample size and data analysis

For the Ghana iLiNS-DYAD Study, an effect size (Cohen's d: difference between group means divided by the pooled standard deviation) of 0.3 (considered a small-to-moderate effect size) (Cohen) was the basis for sample size calculation. Thus, our sample size was based on detecting an effect size of 0.3 between any two groups for any continuous variable at 36 GW, with a two-sided 5% test and 80% power. As described previously (Adu-Afarwuah et al., 2015), we enrolled 1320 pregnant women into the study, but after excluding 177 who received both IFA and MMN supplements during pregnancy because of a temporary mislabeling of supplements, as well as 86 in the LNS group who were pregnant during the same time period, 1,057 women were included in the current analysis. Based on a sample size of 827 women (~275 per group) for whom data were available at 36 GW, we had 94% power to detect an effect size of 0.3 between any two groups for hemoglobin, ZPP, or TfR. This would allow a difference of 3.4 g/L in Hb, 8.9 µmol/mol heme in ZPP, and 0.5 mg/L in TfR (given SD of 11.0, 30.0, and 2.0, respectively) to be detected between any 2 groups.

We posted the statistical analysis plan (www.ilins.org) before analysis. Statistical analysis, by intention-to-treat, was performed using SAS for Windows Release 9.3 (Cary, NC, USA). Background socio-demographic characteristics were summarized as mean ± SD for continuous variables, or number of participants and percentages for categorical variables. As done previously (Adu-Afarwuah et al., 2015), we used 2 indices, namely assets index and housing index as proxy indictors for socioeconomic status, and calculated household food insecurity access (HFIA) score (Coates et al., 2007) as a measure of degree of household food insecurity. Higher values of the assets and housing indices represented higher socioeconomic status, and higher values of the food insecurity index represented higher food insecurity.

We calculated adherence to treatment as percentage of days from enrolment to the home visit closest to the laboratory assessment at 36 GW, when women reported consuming the supplement. We used Hb <100 g/L as our primary definition for low Hb (representing anemia). This was based on previous WHO (WHO, 2007, WHO/UNICEF/UNU, 2001) and International Nutritional Anemia Consultative Group, INACG (Nestel and INACG Steering Committee, 2002) documents that suggest lowering the standard 110 g/L cut-off by 10 g/L for pregnant women of African extraction to achieve adequate sensitivity and specificity for screening purposes (WHO/UNICEF/UNU, 2001). In addition, we defined low Hb using the standard cut-off of Hb <110 g/L, based on a recent WHO recommendation (WHO, 2011) to maintain that cut-off (110 g/L) without any adjustment, because of scarce evidence to support the adjustment. A meta-analysis (Haider et al., 2013) revealed that Hb cut-offs ranging from <100 g/L to 115 g/L have been used in studies to define anemia in pregnant women. We defined high Hb as >130 g/L (Pena-Rosas et al., 2012), elevated ZPP (proxy for iron deficiency) as >60 μmol/mol heme (Walsh et al., 2011) and elevated TfR (proxy for tissue iron deficiency) as > 6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013). Because there is no generally accepted cut-off value for TfR, we derived the 6.0 mg/L cut-off based on the evidence that TfR values obtained using the Automatic Analyzer assay (as used in this study) were on average 30% lower than values obtained with the ELISA assay (Pfeiffer et al., 2007). Therefore, we reduced by 30% the 8.5 mg/L cut-off value used when TfR was determined using ELISA (Vandevijvere et al., 2013) to obtain the cut-off of approximately 6.0 mg/L for our analysis. Because we used 2 cut-offs to define anemia, we also defined iron deficiency anemia (IDA) in two ways: first as Hb <100 g/L and at least one marker of iron deficiency (ZPP > 60 (μmol/mol heme) or TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013)), and second, as Hb <110 g/L and at least one

marker of iron deficiency. We defined elevated inflammatory markers using the cut-off values of >5.0 mg/L for CRP and >1.0 g/L for AGP (Thurnham and McCabe), and categorized women with inflammation as either elevated CRP only (indicative of incubation phase of infection), elevated CRP and AGP (indicative of early convalescence) or elevated AGP only (indicative of late convalescence) (Thurnham and McCabe).

At 36 GW, we calculated overall mean (±SD) values and percentages for Hb and markers of iron status and inflammation. We compared groups by using general linear models (continuous outcomes) and logistic regression models (binary), with Tukey-Kramer adjustment for multiple comparisons. Along with the group comparisons, we calculated pairwise mean differences (continuous outcomes, ANOVA) and relative risks (binary outcomes, Logistic regression) with their 95% CI and p-values. Relative risks were calculated using Poisson regression (Spiegelman and Hertzmark, 2005). In addition, we analyzed changes in the prevalence of anemia, high Hb, and elevated ZPP, TfR, CRP and AGP from enrolment using mixed model logistic regression (SAS PROC GLIMMIX). Where the mixed model logistic regression failed to converge because of sparse data, we used generalized estimating equations model (SAS PROC GENMOD). We analyzed each outcome twice, first without any covariate adjustments, and then with adjustment for covariates significantly associated ($p<0.10$) with the outcome in a bivariate analysis. Because ZPP, TfR, AGP and CRP are not normally distributed, we calculated the group means (± SD or SE), group percentages, and pair-wise mean differences and relative risks with their 95% CI based on untransformed data, but generated the p-values for group or pair-wise comparisons using logarithmically transformed data.

To investigate the possible effect of group differences in adherence to treatment, we performed a per-protocol analysis, which was restricted to women with adherence ≥ 70%. We

evaluated potential interaction of treatment group with pre-specified baseline variables for maternal characteristics, anemia and iron status. These variables were: age, years of schooling, BMI, gestational age at enrolment, household assets index, housing index, food insecurity access score, season at enrolment (dry or wet), primiparous, anemia, and elevated ZPP, TfR, and AGP or CRP. Where an interaction was significant (alpha <0.10), we performed subgroup analysis by including an interaction term between treatment and the effect modifier in the ANCOVA or logistic regression model. For continuous effect modifiers, we used data from all participants to create a linear regression model to predict the values of the outcome at the 10^{th} and 90^{th} percentile of the effect modifier distribution. Each effect modifier was considered separately in the models to avoid collinearity.

In a sensitivity analysis aimed at correcting for the effect of inflammation (CRP and AGP) on the Hb and iron status outcomes, we repeated the above analyses using values of Hb and iron status markers corrected for inflammation (WHO, 2007). These corrected values were calculated by grouping women into 3 inflammation categories, estimating the correction factor (CF) for each inflammation category, and multiplying the Hb and iron status values of each woman by the inflammation category-specific CF (Grant et al., 2012). The 3 inflammation categories were: reference (normal CRP and AGP), incubation (raised CRP and normal AGP), and early (raised CRP and AGP) or late (normal CRP and raised AGP) convalescence [these two phases of convalescence were combined because of small sample sizes and little indication of differences]. For ZPP at 36 GW, women were grouped into 2 inflammation categories (normal versus any inflammation), since the 3-category grouping did not yield consistent results.

Results

We collected data from December 2009 to August 2012. The study flow diagram, as well as the main reasons women were not eligible, or were eligible but not enrolled, were reported elsewhere (Adu-Afarwuah et al., 2015). Among eligible women, those enrolled (n=1320) and those not enrolled (n=606) did not differ in most background characteristics (results not shown). The background characteristics of the 1057 women included in the current analysis are presented in **Table 1**. These characteristics were well-balanced across the three groups. On average, women were about 26 years of age, had about 7 years of formal education, and BMI of about 25 kg/m^3. Nearly all of the women said they were married or living with a partner, slightly more than a third were primiparous, and nearly 10% tested positive for malaria. The average GA at enrolment was 16 weeks. At baseline, we obtained Hb values for all 1057 women, ZPP values for 1055 women, and TfR, CRP and AGP values for 1032 women.

At 36 GW, dropout (4.4%) was low, and did not differ among groups (p = 0.65). Women who dropped out – mainly because of miscarriage (2.8%) and movement from the study site (1.2%) –did not differ in the baseline characteristics from those who were present at 36 GW, except for GA (weeks) at enrolment, which was significantly lower (p = 0.001) for the former (14.5) than for the latter (16.3). Mean (±SD) adherence (% of days from enrolment to the home visit closest to the laboratory assessment at 36 GW when supplement was reportedly consumed) was lower (p=0.001) in the LNS group (68 ± 24) compared to the IFA (74 ± 21) and the MMN (72 ±23) groups. Women usually reported mixing the LNS supplement with porridge, but sometimes mixed it with other foods including soups and stews, or consumed it alone. We obtained Hb values for 827 women, and ZPP, TfR, CRP and AGP values for 822 women; the number (%) of women without Hb values did not differ between groups (p = 0.10). The women

with Hb values did not differ from those without Hb values in most of the baseline characteristics, except that the latter had lower mean housing index (-0.17 ± 1.09 vs. 0.04 ± 0.99; p = 0.011), BMI (24 ± 4.0 vs. 25 ± 4.7 kg/m^2; p = 0.022) and GA at enrolment (16 ± 3.1 vs. 16 ± 3.3 weeks; p = 0.042).

In the intention-to-treat analysis, the overall mean (±SD) values at baseline and 36 GW were 112± 12 and 117 ± 12, respectively, for Hb (g/L), 45 ± 32 and 47 ± 30 for ZPP (μmol/mol heme) and 4.1 ± 2.5 and 4.5 ± 1.7 for TfR (mg/L). From baseline to 36 GW, the prevalence of anemia decreased significantly from 13% to 5.3% (p < 0.001), whilst the reverse was true for the prevalence of high Hb (4.9% vs 12%, p < 0.001) and elevated TfR (9.2% to 15%; p = 0.001), with a moderate change in the prevalence of elevated ZPP (13% vs. 16%; p = 0.06). The mean (±SD) CRP (mg/L) and AGP (g/L) at baseline (6.9 ± 11 and 0.6 ± 0.2, respectively) were slightly greater than at 36 GW (5.7 ± 17 and 0.5 ± 0.2, respectively), which was also reflected in the percentages of women with elevated CRP and AGP at baseline vs. 36 GW (38.2% vs 24.1%; p < 0.001 and 6.3% vs 2.8%; p < 0.001, respectively).

Main group comparisons at 36 gestational weeks

Table 2 shows the unadjusted mean (± SD) Hb (g/L), ZPP (μmol/mol heme), TfR (mg/L), CRP (mg/L) and AGP (g/L) concentrations, by intervention group, at baseline and 36 GW in the intention-to-treat analysis. Baseline values did not differ significantly among groups. At 36 GW, mean Hb was significantly (p < 0.001) greater in the IFA group (120 ±11) than in the LNS (115±12) or MMN (117±12) group; ZPP was significantly (p <0.001) lower in the IFA group (43 ± 30) than in the LNS (50± 29) or MMN (49 ± 30) group; and TfR was significantly (p < 0.001) lower in the IFA group (4.0 ± 1.3) than in the LNS (4.9±1.8) or MMN (4.6±1.7) group. Further

(**Table 3**), the percentage of women with anemia defined either as Hb <100 g/L or Hb <110 g/L was significantly lower in the IFA group compared with the LNS and MMN groups, and when using the latter definition, this percentage was also significantly greater in the LNS compared with the MMN group. Compared with the IFA group, the LNS and MMN groups had greater percentages of women with elevated ZPP (9.4% vs 19% and 19%, respectively; p = 0.003) and elevated TfR (9.0% vs 19% and 15 %, respectively; p = 0.004). Differences among the 3 groups in the prevalence of IDA were marginally significant (p = 0.07) when the Hb cut-off of 100 g/L was used in the definition of IDA, but were significant (p <0.001) when the Hb cut-off of 110 g/L was used. In the latter situation, the risk of IDA was significantly greater in the LNS group compared with the IFA group (p < 0.001), and marginally greater in the MMN compared to the IFA group, and in the LNS compared with the MMN group. The prevalence of high Hb did not differ among groups (p = 0.15).

From baseline to 36 GW, the decrease in the prevalence of anemia (based on our primary definition of Hb <100 g/L) and increase in the prevalence of high Hb were significant for all groups; the change in prevalence over time differed between groups for anemia (*P*-interaction = 0.099) but not for high Hb (*P*-interaction = 0.95). For elevated ZPP, the increase in prevalence from baseline was marginally significant in the LNS group (p = 0.06) and non-significant in the other two groups, and the change in prevalence over time did not differ between groups (*P*-interaction = 0.14). For elevated TfR, the increase in prevalence was significant for the LNS (p = 0.003) and MMN (p = 0.009) groups only, and the change in prevalence over time did not differ between groups (*P*-interaction = 0.17). Apart from the prevalence of anemia defined using the Hb cut-off of 110 g/L, the LNS and MMN groups did not differ significantly in any of the continuous (Table 2) or binary (Table 3) Hb and iron status outcomes.

There were no significant differences among groups in 36 GW mean (±SD) concentrations of CRP (p = 0.98) and AGP (p = 0.35) (Table 2), or the percentages of women in the incubation phase of infection (elevated CRP only; p = 0.26), early convalescence (both CRP and AGP elevated; p = 0.67) or late convalescence (elevated AGP only; p = 1.00). From baseline to 36 GW, the decrease in the percentage of women in the incubation phase of infection was significant for all groups. For percentage of women in early convalescence, only the decrease in the IFA group (p = 0.010) was significant, and for percentage of women in late convalescence (where the mixed model logistic regression did not converge because of sparse data, and hence generalized estimating equations model was used), only the decrease in the MMN group (p = 0.024) was significant.

Adjustments by covariates significantly associated with Hb and the iron status (ZPP and TfR) and inflammatory (CRP and AGP) outcomes (including the baseline value for each outcome) did not alter the unadjusted results (data not shown). In addition, correcting the Hb and iron status values for inflammation in the sensitivity analysis (results not shown) did not change the above findings.

The per-protocol analysis (results not shown) revealed that among women with adherence to treatment ≥ 70% (samples sizes at 36 gestational weeks: 213 in IFA, 222 in MMN and 166 in the LNS group, for Hb), the above findings remained unchanged, except for the fact that the prevalence of anemia (defined as Hb < 100 g/L or < 110 g/L) did not differ between the LNS and MMN groups, and the prevalence of IDA (if definition included Hb < 110 g/L) was greater only in the LNS compared with the IFA group.

Effect modification

346 Interactions of treatment group with BMI, season of enrolment and elevated CRP
347 concentration at baseline were not significant for any of the outcomes. As shown in **Table 4**, the
348 effect of intervention on ZPP concentration at 36 GW was modified by baseline anemia (Hb <
349 100 g/L; *P*-interaction = 0.099), elevated ZPP (*P*-interaction = 0.013) and TfR (*P*-interaction =
350 0.090), GA at enrolment (*P*-interaction = 0.041), and household assets score (*P*-interaction =
351 0.061). Specifically, the difference in mean (±SD) ZPP concentration at 36 GW among
352 intervention groups (MMN and LNS groups compared with the IFA group) was greater among
353 women with anemia at baseline, elevated baseline ZPP, greater GA at enrolment, or lower
354 household assets scores . Similarly, the difference in risk of elevated ZPP among intervention
355 groups was greater in women with greater GA at enrolment (*P*-interaction = 0.057), and elevated
356 TfR at baseline (*P*-interaction = 0.049)
357 The difference in mean TfR concentration at 36 GW among intervention groups was
358 greater among women who were anemic at baseline (*P*-interaction = 0.079), did not have
359 elevated AGP at baseline (*P*-interaction = 0.051), or had greater GA at enrolment (*P*-interaction
360 = 0.016), whereas the difference in risk of elevated TfR among intervention groups was greater
361 in women with less food insecurity.
362 For elevated CRP and AGP at 36 GW, there were significant interactions between
363 intervention group and elevated ZPP at baseline (*P*-interaction = 0.008) and household assets
364 score (*P*-interaction = 0.091), but the stratified analyses (Table 4) did not show consistent results.
365

Discussion

In the iLiNS-DYAD-Ghana study, pregnant women who were provided with standard iron (60 mg) and folic acid supplements from ≤20 GW had significantly greater mean Hb, lower mean ZPP and TfR and lower prevalence of anemia and iron deficiency (elevated ZPP and TfR) at 36 GW than pregnant women who were provided with either the MMN or LNS supplements, both of which contained 20 mg iron. Overall, however, the prevalence of anemia (Hb < 100 g/L) at 36 GW was relatively low (2.2-7.9%), and iron deficiency was evident in <20% of women in all groups. At 36 GW, the three groups did not differ in the percentage of women with high Hb (12.3% overall) or inflammation (CRP and AGP). These findings remained unchanged when analyses were restricted to women who were more adherent to treatment.

A few weaknesses of our study were described previously (Adu-Afarwuah et al., 2015), namely: (a) a fully double-blind study design was not possible because of the physical differences between the SQ-LNS in the form of sachets, and the other two supplements (MMN and IFA) in the form of capsules, and (b) adherence to treatment was assessed by self-report and not direct observation. However, none of the individuals involved in sample collection, laboratory measurements or data analysis had any knowledge of group assignment. The good collaboration with the antenatal clinics (which gave women the confidence to cooperate), relatively low rate of attrition, intense follow-up of participants, and detailed attention we paid to ensuring data quality were notable strengths of the study.

Several possible explanations may be relevant for the observed lower Hb and higher ZPP and TfR values in the MMN and LNS groups compared to the IFA group. First, the 20 mg iron dose used in the MMN and LNS supplements may have been too low for this Ghanaian population of pregnant women. In most (but not all) similar studies, the iron dose of the iron +

folic acid supplement was 60 mg/day and that of the multiple micronutrient supplement was 30 mg/day (Allen and Peerson, 2009, Roberfroid et al., 2011, Mei et al., 2014), although in Indonesia (Suprapto et al., 2002), Mexico (Ramakrishnan et al., 2004), Nepal (Christian et al., 2003) and Tanzania (Makola et al., 2003), both the iron + folic acid and multiple micronutrient supplement groups received iron doses of at least 50 mg/ day. In these studies (even for those that used multiple micronutrients containing 30 mg Fe), pregnant women consuming the multiple micronutrient supplements generally did not differ in Hb or iron status indicators compared to those consuming iron + folic acid (Allen and Peerson, 2009, Mei et al., 2014, Roberfroid et al., 2011). A study of Australian women (Zhou et al., 2009) suggested that 20 mg iron per day may be an adequate dose to prevent iron deficiency anemia during pregnancy compared with higher doses of iron. However, the diet of the women in our sample, as is typical of Ghana, is mainly plant-based and high in phytate (Gibson, 1994), which reduces iron absorption (Baech et al., 2003), so dietary iron needs during pregnancy may be higher in Ghana than in Australia.

Another possibility is that the relatively high dose of zinc (30 mg) in the MMN and LNS supplements may have interfered with iron absorption, as suggested by the results of a supplementation trial in Nepal (Christian et al., 2003). The lack of a good biomarker of zinc status at the individual level makes it difficult to explore this potential mechanism for the differences in iron status between the IFA and other two groups.

It is noteworthy that there were no significant differences in mean Hb or iron status between the LNS and MMN groups, despite the fact that SQ-LNS is a food-based supplement rather than a capsule and some differences in composition could have affected these outcomes. For instance, it is possible that the calcium or phytate in SQ-LNS could have limited the absorption of iron in the LNS group. The lack of differences in Hb and iron status between these

two groups suggests that the iron or other micronutrient content (which was identical in these two supplements, except for the macro-minerals) was the most critical factor.

In Ghana, anemia (Ghana Statistical Service (GSS) et al., 2009) and infections including malaria (Yatich et al., 2009) are common among pregnant women even in relatively high income communities, and evidence (Mockenhaupt et al., 2000) suggests that malaria is a major risk factor for anemia. Thus, it is noteworthy that the prevalence of both anemia and elevated CRP declined significantly in all three groups between baseline and 36 weeks gestation.

It is important to consider the implications of the observed lower mean Hb of the SQ-LNS and MMN groups compared to the IFA group at 36 weeks gestation. Low Hb concentration or anemia in the first or second trimester of pregnancy is linked with poor pregnancy outcomes including low birth weight (Murphy et al., 1986), but no such association has been established for low Hb concentration or anemia in the third trimester (Allen, 2000). Further, Hb concentrations substantially above 110–119 g/L during pregnancy may be independent of iron status and have been linked with poorer health outcomes for the mother and fetus (Yip, 2000, Zhou et al., 1998). Therefore, the higher mean Hb concentrations observed for women in the IFA group compared to those in the LNS and MMN groups in the third trimester (36 GW) may not necessarily be beneficial with respect to birth outcomes. In fact, we previously demonstrated that in this same study (Adu-Afarwuah et al., 2015) the prenatal consumption of LNS (compared to IFA) was associated with greater birth weight, weight-for-age z-score and BMI-for-age z-score, and that, in first-time mothers, prenatal LNS supplementation also increased birth length and head circumference and reduced the proportion of infants with low birth weight, low birth length, and small-for-gestational age. In this cohort, there was no relationship between Hb at 36 wk gestation and infant birth size, and there was actually a significant *negative* relationship between

maternal iron status at 36 wk and birth size (Oaks et al., 2015). Thus, the difference between the LNS and IFA groups in mean maternal Hb, ZPP, and TfR concentrations needs to be weighed against the difference (in the opposite direction) in birth outcomes. In two sets of analyses (Garn et al., 1981, Steer et al., 1995) each involving a large number of pregnant women, the lowest risk of adverse birth outcomes including low birth weight was seen in women with Hb ~95-105 g/L (Steer et al., 1995) or Hb ~100 -110 g/L (Garn et al., 1981). Appropriate cut-offs for ZPP and TfR in pregnancy are not well documented, particularly with respect to functional outcomes. Therefore, it is difficult to judge whether our results should be interpreted as "improvements" in maternal iron status in the IFA group compared to the MMN or LNS group.

We conclude that among pregnant women in a semi-urban setting in Ghana, supplementation with SQ-LNS or MMN containing 20 mg iron resulted in lower Hb and iron status but had no impact on inflammation, when compared with iron (60 mg) plus folic acid (400 µg) treatment. The amount of iron in such supplements that is most effective for improving both maternal Hb/iron status and birth outcomes requires further evaluation.

Key messages

1. In this semi-urban Ghanaian population, the prevalence of anemia (Hb <100 g/L) among pregnant women who received IFA, MMN or SQ-LNS was reduced from 13% at <20 gestational weeks (GW) to 5% at 36 GW.
2. Provision of IFA (with 60 mg Fe) was associated with a greater mean concentration of Hb and lower prevalence of anemia at 36 GW than provision of MMN or SQ-LNS (both with 20 mg Fe).

3. The prevalence of high Hb (>130 g/L) or elevated inflammatory biomarkers (CRP and AGP) at 36 GW was not affected by the type of prenatal supplement provided.
4. More research is needed to determine the concentration of iron in MMN and SQ-LNS supplements that is most effective for improving both maternal Hb/iron status and birth outcomes.

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TABLE 1

Background characteristics of pregnant Ghanaian women whose hemoglobin, and iron status and inflammatory markers were analyzed at 36 gestational weeks, by intervention group[1]

Background characteristics	IFA (N = 349)	MMN (N = 354)	LNS (N = 354)
Age, y	27 ± 5.3 [349]	27 ± 5.7 [354]	27 ± 5.4 [354]
Formal education, y	7.6 ± 3.5 [349]	7.5 ± 3.6 [354]	7.7 ± 3.7 [354]
Body Mass Index, kg/m^2	25 ± 4.3 [342]	25 ± 5.0 [348]	25 ± 4.4 [349]
Low BMI, n/N (%)	11/342 (3.2)	8/348 (2.3)	6/349 (1.7)
Gestational age at enrolment, weeks	16.3 ± 3.3 [346]	16.2 ± 3.2 [353]	16.2 ± 3.3 [349]
Assets index[2]	0.09 ± 0.98 [342]	0.1 ± 0.9 [349]	-.01 ± 0.91 [348]
Housing index[2]	0.04 ± 0.99 [342]	-0.03 ± 1.03 [349]	0.00 ± 1.00 [348]
HFIA Score[3]	2.9 ± 4.6 [345]	2.5 ± 3.9 [346]	2.5 ± 3.9 [348]
Married or co-habiting, n/N (%)	320/349 (91.7)	332/354 (93.8)	328/354 (92.7)
Primiparous women, n/N (%)	131/349 (37.5)	110/354 (31.1)	128/354 (36.2)
Tested positive for malaria[4], n/N (%)	31/349 (8.9)	30/354 (8.5)	40/354 (11.3)

[1] IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group. LNS= Small Quantity Lipid-based Nutrient Supplement group. HFIA is Household Food Insecurity Access Score. N=total number of participants in the group in question; n = number of participants positive on the variable in question; % = percent of participants positive on the variable in question. Values are Mean ± SD [N] or n/N (%).

[2] Proxy indicators for household socioeconomic status; higher values represent higher socioeconomic status.

[3] HFIA (Household food insecurity access) is a proxy indicator for household food insecurity (Coates et al., 2007); higher values represent higher food insecurity

[4] Rapid Diagnostic Test (Clearview Malarial Combo, Vision Biotech, South Africa), which detected *P. falciparum* and non-*P. falciparum* histidine-rich protein-2

TABLE 2

Unadjusted hemoglobin, and iron status and inflammatory markers of pregnant Ghanaian women at baseline and 36 gestational weeks, by intervention group, and pair-wise comparison of groups[1]

Variable	IFA[2] [N=349]	MMN[2] [N=354]	LNS[2] [N=354]	P[3]	Comparison of IFA and MMN		Comparison of IFA and LNS		Comparison of MMN and LNS	
					Mean difference (95 % CI)	p	Mean difference (95 % CI)	p	Mean difference (95 % CI)	p
Hb, g/L										
Baseline	112 ± 13 [349]	111 ± 12 [354]	112 ± 12 [354]		-1 (-4, 1)		0 (-2, 2)		1 (-1, 3)	
36 GW	120 ± 11 [270]	117 ± 12 [291]	115 ± 12 [266]	<0.001	-3 (-6, -1)	0.002	-5 (-7, 3)	<0.001	-2 (-4, 1)	0.18
ZPP, µmol/mol heme										
Baseline	43 ± 28 [347]	46 ± 36 [354]	45 ± 33 [354]		3 (-3, 8)		2 (-4, 8)		-1 (-6, 5)	
36 GW	42 ± 30 [267]	49 ± 30 [291]	50 ± 29 [264]	<0.001	6 (0, 12)	<0.001	7 (1, 13)	<0.001	1 (-5, 7)	0.91
TfR, mg/L										
Baseline	4.0 ± 1.9 [338]	4.0 ± 1.7 [348]	4.3 ± 3.5 [346]		0.0 (-0.4, 0.5)		0.3 (-0.1, 0.8)		0.3 (-0.1, 0.8)	
36 GW	4.0 ± 1.3 [266]	4.6 ± 1.7 [291]	4.9 ± 1.8 [265]	<0.001	0.5 (0.2, 0.9)	<0.001	0.8 (0.5, 1.2)	<0.001	0.3 (-0.0, 0.6)	0.07
CRP, mg/L										
Baseline	7.9 ± 14 [338]	5.8 ± 8.3 [348]	6.9 ± 11 [346]		-2.1 (-4.1, -0.1)		-1.1 (-3.1, 1.0)		1.0 (-1.0, 3.0)	
36 GW	5.6 ± 18 [266]	5.7 ± 14 [291]	5.9 ± 18 [265]	0.85	0.1 (-3.3, 3.5)	0.95	0.3 (-3.2, 3.8)	0.97	0.2 (-3.2, 3.6)	0.84
AGP, g/L										
Baseline	0.7 ± 0.2 [338]	0.6 ± 0.2 [348]	0.6 ± 0.2 [346]		0.0 (-0.1, 0.0)		0.0 (-0.1, 0.0)		0.0 (0.0, 0.0)	

36 GW	0.5 ± 0.2 [266]	0.5 ± 0.2 [291]	0.5 ± 0.2 [265]	0.30	0.0 (0.0, 0.1)	0.65	0.0 (0.0, 0.0)	0.80	0.0 (-0.1, 0.0)	0.27

[1]IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group; LNS= Small Quantity Lipid-based Nutrient Supplement group. AGP, CRP, GW, Hb, TfR, and ZPP are alpha-1 acid glycoprotein, C-reactive protein, gestational weeks, hemoglobin, transferrin receptor, and zinc protoporphyrin, respectively. Analyses are based on ANOVA (SAS, PROC GLM). Group means (±SD) and pair-wise mean difference (95% CI) were calculated using untransformed data. Except for Hb, all p-values for group or pair-wise comparisons were generated from log-transformed data; untransformed data were used for comparisons of mean Hb values. N=total number of participants in the group in question.

[2] Values are Mean ± SD [N].

[3]P-values, with Tukey-Kramer adjustments, compare all three groups.

TABLE 3

Number (percentage) of pregnant Ghanaian women with abnormal hemoglobin, and iron status and inflammatory markers at baseline and 36 weeks of gestation, by intervention group and pairwise relative risks (RR) between groups[1]

	IFA[2] [N=349]	MMN[2] [N=354]	LNS[2] [N=354]	P[3]	Comparison of IFA and MMN[4]		Comparison of IFA and LNS[4]		Comparison of MMN and LNS[4]	
					RR (95 % CI)	p	RR (95 % CI)	p	RR (95 % CI)	p
Low Hb (< 100 g/L)[5]										
Baseline	39/349 (11.2)	51/354 (14.4)	47/354 (13.3)		1.29 (0.81, 2.05)		1.19 (0.74, 1.91)		0.92 (0.59, 1.43)	
36 GW	6/270 (2.2)	17/291 (5.8)	21/266 (7.9)	0.019	2.63 (0.88, 7.86)	0.09	3.55 (1.22, 10.3)	0.013	1.35 (0.65, 2.83)	0.60
Low Hb (< 110 g/L)[6]										
Baseline	139/349 (39.8)	157/354 (44.4)	134/354 (37.9)		1.11 (0.90, 1.37)		0.95 (0.76, 1.19)		0.85 (0.69, 1.06)	
36 GW	38/270 (14.1)	69/291 (23.7)	88/266 (33.1)	<0.001	1.68 (1.10, 2.59)	0.011	2.35 1.56, 3.53)	<0.001	1.40 (1.01, 1.92)	0.038
High Hb[7]										
Baseline	21/349 (6.0)	15/354 (4.2)	16/354 (4.5)		0.70 (0.33, 1.52)		0.75 (0.35, 1.60)		1.07 (0.47, 2.43)	
36 GW	42/270 (15.6)	32/291 (11.0)	28/266 (10.5)	0.15	0.71 (0.42, 1.18)	0.25	0.68 (0.40, 1.16)	0.20	0.96 (0.54, 1.70)	0.98
Elevated ZPP[8]										
Baseline	40/347 (11.5)	54/354 (15.3)	46/354 (13.0)		1.32 (0.84, 2.09)		1.13 (0.70, 1.81)		0.85 (0.55, 1.32)	
36 GW	25/267 (9.4)	56/291 (19.2)	49/264 (18.6)	0.003	2.06 (1.21, 3.48)	0.003	1.98 (1.16, 3.40)	0.007	0.96 (0.64, 1.46)	0.98
Elevated TfR[9]										
Baseline	29/338 (8.6)	29/348 (8.3)	37/346 (10.7)		0.97 (0.54, 1.75)		1.25 (0.72, 2.17)		1.28 (0.74, 2.23)	
36 GW	24/266 (9.0)	44/291 (15.1)	51/265 (19.2)	0.004	1.68 (0.96, 2.94)	0.08	2.13 (1.24, 3.67)	0.003	1.27 (0.82, 1.97)	0.40
IDA[10]										
Baseline	20/338 (5.9)	22/348 (6.3)	22/346 (6.4)		1.07 (0.53, 2.15)		1.07 (0.53, 2.17)		1.01 (0.51, 1.99)	
36 GW	2/264 (0.8)	11/291 (3.8)	11/263 (4.2)	0.07	4.99 (0.83, 29.9)	0.09	5.52 (0.92, 33.1)	0.06	1.11 (0.42, 2.95)	0.97
IDA[11]										
Baseline	36/338 (10.7)	37/348 (10.6)	37/346 (10.7)		1.00 (0.59, 1.68)		1.00 (0.60, 1.69)		1.01 (0.60, 1.68)	
36 GW	12/264 (4.5)	27/291 (9.3)	41/263 (15.6)	<0.001	2.04 (0.93, 4.49)	0.08	3.43 (1.63, 7.20)	<0.001	1.68 (0.97, 2.90)	0.07
Elevated CRP only[12]										

Baseline	113/338 (33.4)	108/348 (31.0)	121/346 (35.0)		0.93 (0.72, 1.20)		1.05 (0.82, 1.34)		1.13 (0.87, 1.45)	
36 GW	49/266 (18.4)	70/291 (24.1)	59/265 (22.3)	0.26	1.31 (0.89, 1.93)	0.24	1.21 (0.81, 1.81)	0.52	0.93 (0.64, 1.33)	0.87
Elevated CRP+AGP[13]										
Baseline	26/338 (7.7)	15/348 (4.3)	11/346 (3.2)		0.56 (0.27, 1.17)		0.41 (0.18, 0.94)		0.74 (0.30, 1.84)	
36 GW	7/266 (2.6)	9/291 (3.1)	5/265 (1.9)	0.67	1.18 (0.37, 3.77)	0.94	0.72 (0.18, 2.79)	0.83	0.61 (0.17, 2.22)	0.64
Elevated AGP only[14]										
Baseline	3/338 (0.9)	5/348 (1.4)	5/346 (1.4)		1.62 (0.30, 8.88)		1.63 (0.30, 8.93)		1.01 (0.23, 4.38)	
36 GW	1/266 (0.4)	0/291 (0.0)	1/265 (0.4)	1.00						

[1]IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group; LNS=Small Quantity Lipid-based Nutrient Supplement group. AGP, CRP, GW, Hb, IDA, TfR, and ZPP are alpha-1 acid glycoprotein, C-reactive protein, gestational weeks, hemoglobin, iron deficiency anemia, transferrin receptor, and zinc protoporphyrin, respectively. N=total number of participants in the group in question.

[2]Values are number of participants positive on the variable in question/N (% of participants positive on the variable in question).

[3]P-vaues compare all three groups, with Tukey-Kramer adjustment, using logistic regression (SAS PROC LOGISTIC).

[4]Relative Risks, RR(95% CI) and their p-values are based on Poisson regression (Spiegelman and Hertzmark, 2005).

[5] Nestel and INACG Steering Committee, 2002, WHO, 2007, WHO/UNICEF/UNU, 2001.

[6] WHO, 2011.

[7]Hb >130 g/L (Pena-Rosas et al., 2012).

[8]ZPP > 60.0 (µmol/mol heme).

[9]TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013).

[10]Hb <100 g/L (Nestel and INACG Steering Committee, 2002, WHO, 2007, WHO/UNICEF/UNU, 2001) and at least one marker of iron deficiency (ZPP > 60.0 (µmol/mol heme) or TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013)).

[11]Hb <110 g/L (WHO, 2011) and at least one marker of iron deficiency (ZPP > 60.0 (µmol/mol heme) or TfR >6.0 mg/L (Pfeiffer et al., 2007, Vandevijvere et al., 2013)).

[12]CRP >5.0 mg/L and AGP not >1.0 g/L (Thurnham and McCabe).

[13] CRP >5.0 mg/L and AGP >1.0 g/L (Thurnham and McCabe).

[14] CRP not > 5.0 mg/L and AGP >1.0 g/L (Thurnham and McCabe).

TABLE 4

Effect of intervention on iron status and inflammatory outcomes of pregnant Ghanaian women, stratified by baseline characteristics[1]

Outcomes	IFA[2]	MMN[2]	LNS[2]	P[3]	P[4]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
						Difference or RR	P	Difference or RR	P	Difference or RR	P
ZPP, μmol/mol heme[5]											
Baseline anemia				0.099			.		.		.
No[†]	44 ± 2 [233]	47 ± 2 [245]	48 ± 2 [226]		0.09	3 (-2, 9)	0.34	5 (-0, 11)	0.08	2 (-4, 8)	0.71
Yes	36 ± 6 [26]	54 ± 5 [41]	53 ± 5 [33]		0.013	18 (3, 34)	0.017	18 (1, 34)	0.032	-1 (-16, 14)	0.99
Elevated baseline ZPP				0.013			.		.		.
No	42 ± 2 [230]	46 ± 2 [242]	46 ± 2 [226]		0.19	3 (-2, 9)	0.34	4 (-2, 10)	0.20	1 (-5, 7)	0.94
Yes	47 ± 5 [29]	64 ± 5 [44]	72 ± 5 [33]		0.001	18 (3, 32)	0.015	25 (9, 41)	0.001	8 (-7, 22)	0.43
Elevated baseline TfR				0.090			.		.		.
No	43 ± 2 [239]	47 ± 2 [261]	49 ± 2 [233]		0.06	4 (-2, 9)	0.28	6 (0, 12)	0.048	2 (-4, 8)	0.65
Yes	41 ± 6.5 [20]	63 ± 6 [25]	59 ± 6 [26]		0.017	22 (3, 41)	0.017	18 (-1, 36)	0.07	-4 (-22, 14)	0.84
GA at enrolment				0.041			.		.		.
At 10th percentile[‡]	46 ± 3	51 ± 3	44 ± 3		0.28	5 (-5, 15)	0.44	-1 (-11, 9)	0.97	-6 (-16, 4)	0.29
At 90th percentile	40 ± 3	48 ± 3	54 ± 3		0.002	7 (-1, 16)	0.12	13 (5, 22)	0.001	6 (-3, 15)	0.25
Assets score				0.061			.		.		.
At 10th percentile	40 ± 3	46 ± 3	54 ± 3		0.003	5 (-4, 15)	0.38	14 (4, 24)	0.002	8 (-1, 18)	0.11
At 90th percentile	46 ± 3	50 ± 3	46 ± 3		0.37	5 (-4, 13)	0.44	-0 (-9, 9)	1.00	-5 (-14, 4)	0.46
Elevated ZPP[6]											
GA at enrolment				0.057			.		.		.
At 10th percentile[§]	11.1 (5.7, 20.5)	23.2 (15.3, 33.6)	11.6 (6.4, 20.0)		0.06	3.0 (0.9, 4.6)	0.12	1.0 (0.4, 2.5)	1.00	0.5 (0.2, 1.1)	0.11
At 90th percentile	5.8 (2.9, 11.4)	14.9 (9.6, 22.3)	20.7 (14.1, 29.2)		0.004	2.4 (1.0, 5.7)	0.049	3.4 (1.5, 7.7)	0.003	1.4 (0.8, 2.5)	0.46

Outcomes	IFA[2]	MMN[2]	LNS[2]	P[3]	P[4]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
						Difference or RR	P	Difference or RR	P	Difference or RR	P
Elevated baseline TfR				0.049			.		.		.
No[¶]	17/234 (7.4)	44/256 (17.4)	32/229 (14.0)		0.008	2.2 (1.2, 4.0)	0.006	1.8 (1.0, 3.4)	0.08	0.8 (0.5, 1.3)	0.61
Yes	1/20 (3.0)	3/25 (10.0)	8/25 (31.3)		0.014	1.3 (0.4, 4.6)	0.41	2.7 (0.8, 9.4)	0.016	2.1 (0.7, 6.1)	0.14
TfR, mg/L[7]											
Baseline anemia				0.079			.		.		.
No	4.1 ± 0.1 [232]	4.5 ± 0.1 [245]	4.8 ± 0.1 [226]		<0.001	0.4 (0.1, 0.8)	0.004	0.7 (0.4, 1.0)	<0.001	0.3 (-0.0, 0.6)	0.11
Yes	3.5 ± 0.3 [26]	4.8 ± 0.3 [41]	5.0 ± 0.3 [33]		<0.001	1.3 (0.4, 2.1)	0.002	1.5 (0.6, 2.4)	<0.001	0.2 (-0.6, 1.1)	0.76
Elevated baseline AGP				0.051			.		.		.
No	4.1 ± 0.1 [238]	4.6 ± 0.1 [272]	4.9 ± 0.1 [250]		<0.001	0.6 (0.3, 0.9)	<0.001	0.8 (0.5, 1.2)	<0.001	0.3 (-0.02, 0.6)	0.07
Yes	4.0 ± 0.4 [20]	4.0 ± 0.4 [14]	3.3 ± 0.5 [9]		0.503	-0.0 (-1.2, 1.2)	1.00	-0. 7 (-2.1, 0.8)	0.51	-0.66 (-2.2, 0.8)	0.56
GA at enrolment				0.016			.		.		.
At 10th percentile	4.4 ± 0.2	5.0 ± 0.2	4.8 ± 0.2		0.023	0.9 (0.1, 1.1)	0.019	0.4 (-0.1, 0.9)	0.16	-0.2 (-0.7, 0.3)	0.64
At 90th percentile	3.8 ± 0.1	4.3 ± 0.1	4.9 ± 0.1		<0.001	0.5 (0.0, 0.9)	0.041	1.1 (0.7, 1.6)	<0.001	0.7 (0.2, 1.1)	0.002
Elevated TfR[8]											
HFIA score				0.047			.		.		.
At 10th percentile	5.3 (2.9, 9.7)	15.7 (10.8, 22.2)	15.3 (10.5, 21.7)		0.004	2.3 (1.1, 4.7)	0.006	2.5 (1.2, 5.0)	0.007	1.1 (0.6, 1.8)	1.00
At 90th percentile	9.7 (5.1, 17.5)	4.9 (1.7, 13.5)	18.7 (10.3, 31.5)		0.06	0.7 (0.2, 2.0)	0.50	1.9 (0.8, 4.6)	0.27	2.8 (0.9, 8.7)	0.06
Elevated CRP[9]											
Elevated baseline ZPP				0.008			.		.		.
No	41/225 (18.3)	72/238 (30.1)	47/221 (21.4)		0.013	1.6 (1.1, 2.4)	0.014	1.2 (0.8, 1.8)	0.71	0.7 (0.5, 1.1)	0.11
Yes	9/29 (30.1)	6/43 (13.5)	11/33 (32.0)		0.09	0.5 (0.2, 1.1)	0.17	1.1 (0.5, 2.1)	0.99	2.1 (0.9, 4.9)	0.10

Outcomes	IFA[2]	MMN[2]	LNS[2]	P[3]	P[4]	Comparison of MMN and IFA (n = 349)		Comparison of LNS and IFA (n = 354)		Comparison of LNS and MMN (n = 354)	
						Difference or RR	P	Difference or RR	P	Difference or RR	P
Elevated AGP[10]											
Assets score				0.091			.		.		.
At 10th percentile	1.0 (0.2, 4.8)	1.0 (0.2, 4.5)	2.8 (0.9, 8.4)		0.39	1.0 (0.1, 9.8)	1.00	2.8 (0.6, 14.1)	0.51	2.8 (0.3, 28.0)	0.48
At 90th percentile	4.3 (1.5, 11.3)	4.4 (1.7, 10.8)	0.7 (0.1, 3.9)		0.14	1.0 (0.2, 4.5)	1.00	0.16 (0.0, 1.3)	0.16	0.2 (0.0, 1.4)	0.14

[1]IFA= Iron-Folic Acid group; MMN=Multiple Micronutrients group; LNS=Small Quantity Lipid-based Nutrient Supplement group. GA, Hb, HFIA, TfR, and ZPP are gestation age, hemoglobin, household food insecurity access, transferrin receptor, and Zinc protoporphyrin, respectively. Baseline anemia is Hb <100 g/L (Nestel and INACG Steering Committee, 2002, WHO, 2007, WHO/UNICEF/UNU, 2001). Analyses are based on ANCOVA (SAS PROC MIXED, with SLICE option) for continuous outcomes or logistic regression (SAS PROC GLIMMIX, with SLICE option) for binary outcomes. Linear regression modeling was used to predict the outcome at the 10th and 90th percentile of continuous baseline effect modifiers.

[2]Values are mean ± SE [total number of participants], or mean ± SE, or percent of participants positive on the variable in question (95% CI) or number of participants positive on the variable in question/total number of participants (percent of participants positive on the variable in question). Values are adjusted for variables significantly associated with the outcome variable in bivariate analysis.

[3]P-values are for interaction with the outcome in question

[4]P-values compare all three groups in each stratum.

[5]ZPP values are adjusted for age, parity, season at enrolment, and baseline Hb, ZPP, and TfR concentrations.

[6]Elevated ZPP (ZPP >60 µmol/mol heme) adjusted for age, education, and baseline Hb, ZPP and TfR concentrations.

[7]TfR values are adjusted for age, season at enrolment, and baseline Hb, ZPP, TfR and AGP concentrations.

[8]Elevated TfR (TfR > 6.0 mg/L) adjusted for age, gestational age enrolment, season at enrolment, and baseline Hb, ZPP, and TfR concentrations.

[9]Elevated CRP (CRP > 5.0 mg/L) adjusted for BMI, household food insecurity access score, and baseline ZPP, AGP and CRP concentrations.

[10]Elevated AGP (AGP > 1.0 g/L) adjusted for age, parity, and season as enrolment.

[†]Values are group mean ± SE [number of participants], and difference in means (95% CI) and p-values. All such values.

[‡]Values are group mean ± SE, and difference in means (95% CI) and p-values. All such values.

[§]Values are group % (95% CI), and relative risk (95% CI) and p-values. All such values.

[¶]Values are number of participant (%), and relative risk (95% CI) and p-values. All such values.